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Identification of two novel elements involved in human MUC1 gene expression *in vivo*

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Abstract

Background: MUC1, a membrane-tethered glycoprotein that is expressed on a number of epithelial cell types *in vivo*, is over-expressed in adenocarcinomas and thought to play a significant role in tumour progression and metastasis. Hence, elucidation of the mechanisms of regulation of *MUC1* gene expression is of considerable biological importance. Our aim was to evaluate regulation of *MUC1* expression *in vivo*.

Materials and Methods: DNase I hypersensitive sites (DHS) were mapped in chromatin from human cell lines and human *MUC1* transgenic mice. *MUC1* expression was evaluated by RT-PCR and Northern blots.

Results: We identified two novel DHS in the *MUC1* promoter at −750 bp and −250 bp from the transcriptional start site. These DHS were detected in human cell lines and in a human *MUC1* transgene in mice. The −750 DHS was apparent in many cell types irrespective of the level of *MUC1* expression but the −250 DHS was only evident in cells that express *MUC1* and its intensity correlated with the abundance of *MUC1* transcripts. The −250 DHS became undetectable in cell lines representing a transition from colon adenoma to carcinoma, commensurate with a significant reduction in *MUC1* expression.

Conclusions: The −750 and −250 regions are conserved between the human *MUC1* and mouse *Muc1* genes and may be associated with functionally important genetic elements. The DHS at −250 is in the vicinity of previously defined purine/pyrimidine mirror repeat elements that may form intramolecular H-DNA structures, which can alter the accessibility of chromatin to regulatory proteins.

Introduction

MUC1 is a cell surface-associated mucin glycoprotein that is highly overexpressed and differentially glycosylated by various adenocarcinomas (1). The MUC1 protein plays a role in the biological properties of tumour progression, especially the process of metastasis.

The promoter of the *MUC1* gene has been partially characterized and some *cis* elements that are important for basal promoter activity have been identified (2–4). Sequential deletions, specific deletions and site specific mutations of sequences 5′ to the *MUC1* gene revealed that at least 600 bp of upstream sequence was required for maximal promoter activity in transient transfections (2,3). This effect may be partly due to deletion of an Sp1 site located approximately −570 bp from the transcription start site, an AP-3 site adjacent to this, or several other potential *cis* elements just downstream. DNase I footprint and/or gel shift analysis revealed several putative elements in the 5′ region that may be bound by protein factors. Based on these results, two different regions have been postulated to be involved in the basal control of expression of the *MUC1* gene. One element, E-MUC1, located immediately upstream of the TATA box, within 100 bp of the transcription start site, may bind specific transcription-regulating factors responsible for determining the tissue-specific expression of the *MUC1* gene (3). A second region between −485 and −505 appears to be involved in the regulation of transcription of the *MUC1* gene (2). This DNA sequence binds to a 45 kDa protein present in breast carcinoma cell line, MCF7, and acts in an orientation-dependent fashion in reporter constructs. In addition, a secreted factor capable of stimulating the production of MUC1 by human colon carcinoma cells has been described (5). This protein, called mucomodulin, has an apparent molecular mass of 70 kDa (4) and was observed to stimulate the production of MUC1 in greater than 70% of colon carcinoma cell lines tested. A subsequent study identified a *cis* element, called RME, in the flanking 5′ region of the *MUC1* gene that renders CAT constructs responsive to treatment with mucomodulin (4). The Sp1 transcription factor has also been implicated in cell type specific transcription of *MUC1* through several binding sites in the promoter (3,6). Further, *Muc1* expression has been shown be hormonally regulated (7).

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The regulatory mechanisms that result in overexpression of *MUC1* in tumours have not been established. Activation of Signal Transducer and Activator of Transcription (STATs) proteins are probably important because the *MUC1* promoter contains a functional STAT3/1 element that is responsive to IL-6 and γ-interferon in reporter gene assays (8). In addition, *MUC1* expression in human mammary cell lines is regulated by the c-ErbB2 and ras signalling pathways (9).

Our aim was to search *in vivo* for novel regulatory elements within the *MUC1* gene and its 5′ and 3′ flanking regions by investigating DNase I hypersensitive sites (DHS), which are often associated with these elements. DHS were evaluated in human cell lines that express or do not express the gene, in a colonic cell line showing progression from adenoma to carcinoma (10) and in transgenic mice carrying a human *MUC1* genomic construct. The transgenic mice have a 10.6 kb *Sac* II fragment of genomic DNA that includes 1.6 kb of upstream sequence and 1.9 kb of downstream sequence (11) and show tissue-specific expression of *MUC1* similar to that seen in humans (12).

We defined two novel DNase I hypersensitive sites in the *MUC1* promoter. These two DHS are detected *in vivo* in cell lines and in certain tissues from *MUC1* transgenic mice that express the human transgene. Further, the appearance of one of these DHS correlates with high levels of *MUC1* transcription in certain carcinoma cell lines. This DHS may be associated with a regulatory element that causes elevated *MUC1* transcription in some primary cell types and tumour cell lines.

Materials and Methods

Cell Culture

The following cell lines were used; HPAF (13), Caco2 (14), HT29 (15) and MCF7 (16) were cultured in DMEM; the lymphoblastoid cell line 37566 was cultured in RPMI 1640 and the AA/C1 and AA/C1/SB10C colonic cell lines in DMEM supplemented with 1 μg/ml hydrocortisone and 0.2 units/ml insulin (10).

Transgenic Mice

The generation of C57/BL6 mice carrying the 10.6 kB *Sac* II fragment of the human *MUC1* gene are described elsewhere (11,12). Control mice were wild type C57/BL6. Mouse tissues were collected immediately after death and either placed in liquid nitrogen for RNA extraction or processed directly for chromatin extraction.

Extraction of Chromatin

Chromatin was extracted from transgenic and normal mouse tissues and from cell lines as described previously (17–19). For all tissues chromatin was extracted from the whole organ without further microdissection.

RNA Extraction, Reverse Transcriptase-PCR (RT-PCR) and Northern Blots

Total RNA was extracted from transgenic and normal mouse tissues and from cell lines by standard methods (20). All tissues were evaluated for transcription of human *MUC1* and mouse *Muc1* mRNA by standard methods of RT-PCR (Superscript). PCR parameters were 95°C 1 min, 50°C 2 min, 72°C 5 min for 30 cycles. The locations of primers used for RT-PCR were MUC1HMA 5′ ACTACTACCAAGAGCTG 3′ (J05582: 3264–3280; M84683: 1339–1355) and MUC1HMB 5′ CTCATAGGATGGTAGGT3′ (J05582: 3693–3677; M84683: 1762–1746). The 429 bp human cDNA product is cleaved by *Dra* III into 273 and 156 bp fragments and the 424 bp murine cDNA product by *Eag* I into 261 and 163 bp fragments. These data are not truly quantitative as the MUC1HMA primer, though matching the human *MUC1* sequence exactly has a 1 base mismatch with the murine gene. The MUC1HMB primers are 100% matched to human and mouse gene sequences. The β-actin primers were β 3′ ATGCCATCCTGCGTCTGGACCTGGC and β 5′ AGCATTTGCGGTGCGACATGGAGGG producing a 607 bp fragment from mouse RNA. RT-PCR for *MUC1* expression from human cell lines was carried out as described previously (21). Northern blots of total RNA from each cell line were carried out by standard methods and were probed with 5′MUC1 probe described below.

DNase I Hypersensitivity Assays

Chromatin from mouse tissues and cell lines was probed for DNase I hypersensitive regions by standard methods (22). The probes used for DHS Southern blots were 5′MUC1 (bases 1–345 of the *MUC1* cDNA), PB352 a 352 base pair *Pvu* I/*Bam*H I fragment of the *MUC1* cDNA (J05582: 3748–4100), BS308 (*Bam*H I/*Sac* I M61170: 6633–6941), AB350 (*Afl* II/*Bsm* I M61170: 3255–3600) and BS280 (*Bsp*LU11 I/*Sac* I U16175: 4675–4953). AB350 hybridized to human genomic DNA only and not to mouse genomic DNA under the conditions used and BS280 hybridized to mouse but not human DNA.

Results

A number of previous studies have been carried out on the *MUC1* promoter *in vitro*. Important sequence motifs identified by the previous studies in the 5′ region of the *MUC1* gene are shown in Fig. 1.

Identification of DHS in the MUC1 Gene in Human Cell Lines

The *MUC1* coding region is flanked by *Bam*H I sites at −2239 (M61170: 632) with respect to the transcriptional start site and within the 3′ untranslated region (M61170:6633). This *Bam*H I fragment of about 8 kb (the sequence of M61170 predicts a 6 kb *Bam*H I fragment due to polymorphisms in the length of the *MUC1* tandem repeat) and the adjacent 3′*Bam*H I

Fig. 1. The sequence of the immediate 5′ promoter region of the human *MUC1* gene. (from X69118) Important sequence motifs previously described by others and the novel DHS are shown.

fragment were probed for DHS using the 5′MUC1, PB352 and BS308 probes shown in Figure 2A. Two DHS were identified in the 5′ promoter region of MUC1 in chromatin from the HPAF pancreatic adenocarcinoma cell line. Figure 2B shows an 8 kb *Bam*H I genomic fragment hybridising to the 5′MUC1 probe and subfragments at 6.5 and 6 kb that correspond to DHS at about −750 bp and −250 bp with respect to the transcriptional start site. *Bsr*G I (x69118: 2520) and *Sac* I (x69118: 2154) cleavage sites at −351 and −717 respectively from the transcription start site enable confirmation of the approximate location of the DHS by the *Bam*H I/*Sac* I and *Bam*H I/*Bsr*G I double digestion (Fig. 2). These DHS were also seen with the PB352 probe. Screening of DNA lying 3′ to the *Bam*H I site at M61170:6633 failed to reveal any additional DHS in HPAF or lymphoblastoid (37566) cell line chromatin.

Additional cell lines were evaluated to establish whether the −750 and −250 DHS were seen only in chromatin from the HPAF pancreatic adenocarcinoma or were a more general phenomenon. Unlike in HPAF where only 1 genomic *Bam*H I fragment hybridises to the 5′MUC1 probe, as the cell line is homozygous or hemizygous for *MUC1* (Fig. 3A), all other cell lines analysed showed 2 genomic fragments presumably due to variation in the *MUC1* tandem repeat number on the 2 alleles. Chromatin isolated from the breast carcinoma cell line MCF7 contained the same two DHS though at a reduced intensity (Fig. 3B). The −750 bp site was also seen in the lymphoblastoid cell line (37566) (Fig. 3C) and the colon carcinoma cell line HT29 (data not shown).

Evaluation of the −750 and −250 DHS in a Colon Adenoma to Carcinoma Transition

The AA/C1 (adenoma) and AA/C1/SB10C (carcinoma) cell lines represent models of cancer progression in the human colon (10). The AA/C1 line is a premalignant variant of a human colonic adenoma cell line PC/AA derived from a familial polyposis coli patient. The AA/C1/SB10C line was generated by treating AA/C1 with agents that induce differentiation and carcinogenesis. Though *MUC1* is expressed at a low levels in normal human colonic

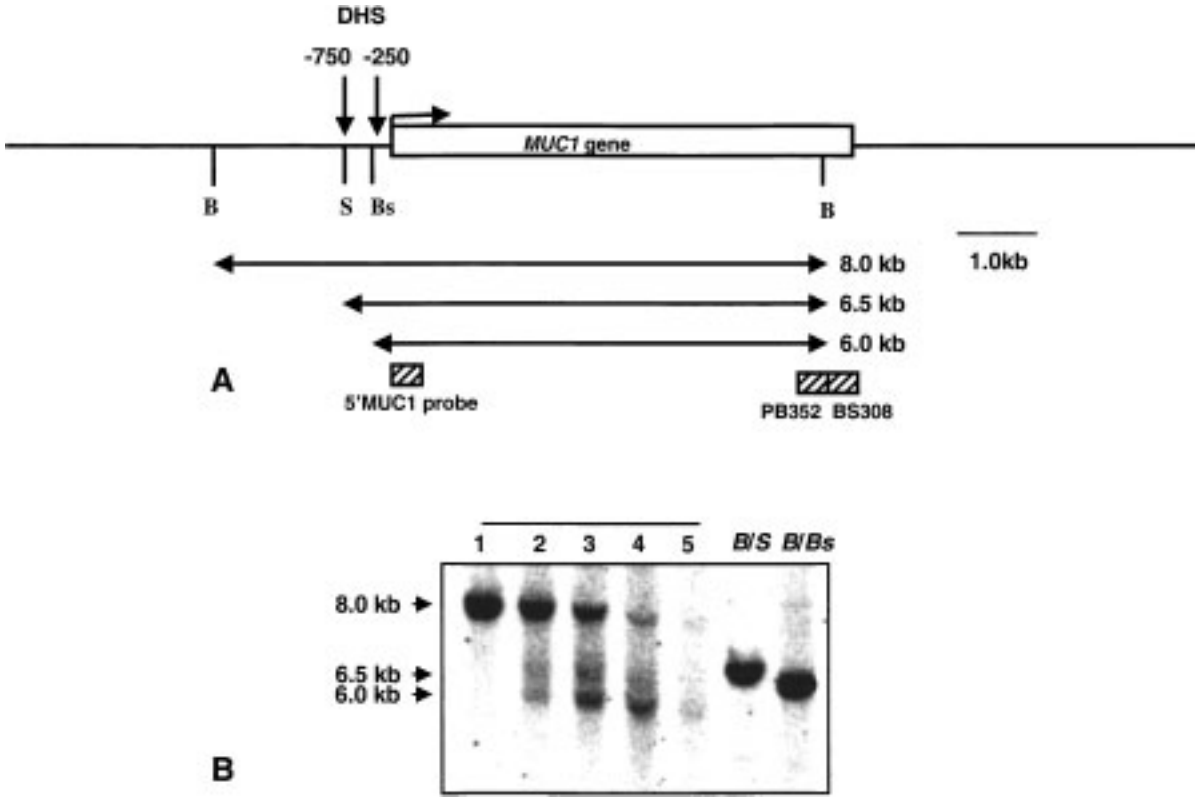

Fig. 2. DNase I hypersensitive sites at −750 bp and −250 bp with respect to the transcriptional start site of the human *MUC1* gene in HPAF chromatin. (A) Map of region. Arrows above the line denote the DHS positions. Restriction sites shown below the line are B = *Bam*H I, Bs = *Bsr*G I, S = *Sac* I. Below the line are shown the probes (hatched) and the subfragments produced by DHS. **(B)** Southern blot of DNase I digested HPAF chromatin cleaved with *Bam*H I and hybridised with 5′MUC1 probe. Lanes 1 (0°C) and 2 (37°C) show DNA prepared from nuclei which had not been treated with DNase I. Lanes 3–5 show DNA prepared from nuclei treated with increasing amounts of DNase I; (Lane 3, 20 units; Lane 4, 40 units; Lane 5, 80 units DNase I). The detection of the DHS in lane 2 is probably due to endogenous DNase I activity at 37°C.

epithelium and colon carcinoma-derived cell lines (23,24) these 2 cell lines have been shown to express MUC1 glycoprotein (25). Evaluation of the −750 and −250 DHS in these 2 cell lines showed both DHS in the adenoma (AA/C1) (Fig. 3D) while only the −750 DHS was evident in the carcinoma cell line (AA/C1/SB10C) (Fig. 3E).

Expression of the Human MUC1 Gene in Human Cell Lines

There have been many reports on the relative expression levels of MUC1 mRNA in different cell lines. To evaluate the potential significance of our data on the presence of the −750 and −250 DHS all cell lines were tested by a semi-quantitative RT-PCR assay for *MUC1* and by northern analysis. Fig. 4A shows RT-PCR data for MUC1 mRNA expression (656 bp product) relative to a housekeeping gene β glucocerebrosidase (572 bp product). As expected high levels of MUC1 mRNA are seen in the HPAF line and MCF7 while the *MUC1* gene expression is barely detectable in the lymphoblastoid cell line (37566). *MUC1* expression in the AA/C1 cell line is significantly less than in HPAF and further reduced in the AA/C1/SB10C cell line. These data were confirmed and extended by northern analysis (Fig. 4B), which showed that the levels of MUC1 mRNA in HPAF are significantly higher than in MCF7, and that MUC1 mRNA was undetectable in the 37566, AA/C1 and AA/C1/SB10C cell lines.

Presence of the −750 and −250 DHS Correlates with MUC1 Expression

The −750 DHS was weakly evident in all cell lines analysed irrespective of *MUC1* expression levels. In contrast, presence of the −250 DHS correlated with *MUC1* expression levels. The −250 DHS was not detected in the lymphoblastoid cell line (37566) in which very low levels of MUC1 mRNA were seen by RT-PCR. In MCF7, which expresses moderate amounts of MUC1 mRNA, the −750 and −250 DHS are of approximately equal intensity. In HPAF, which expresses very high levels of MUC1 mRNA the −250 DHS is of much greater intensity than the −750 DHS. In cells representing the adenoma (AA/C1) to carcinoma (AA/C1/SB10C) transition, which is accompanied by a decrease in MUC1 mRNA, the −250 DHS disappears.

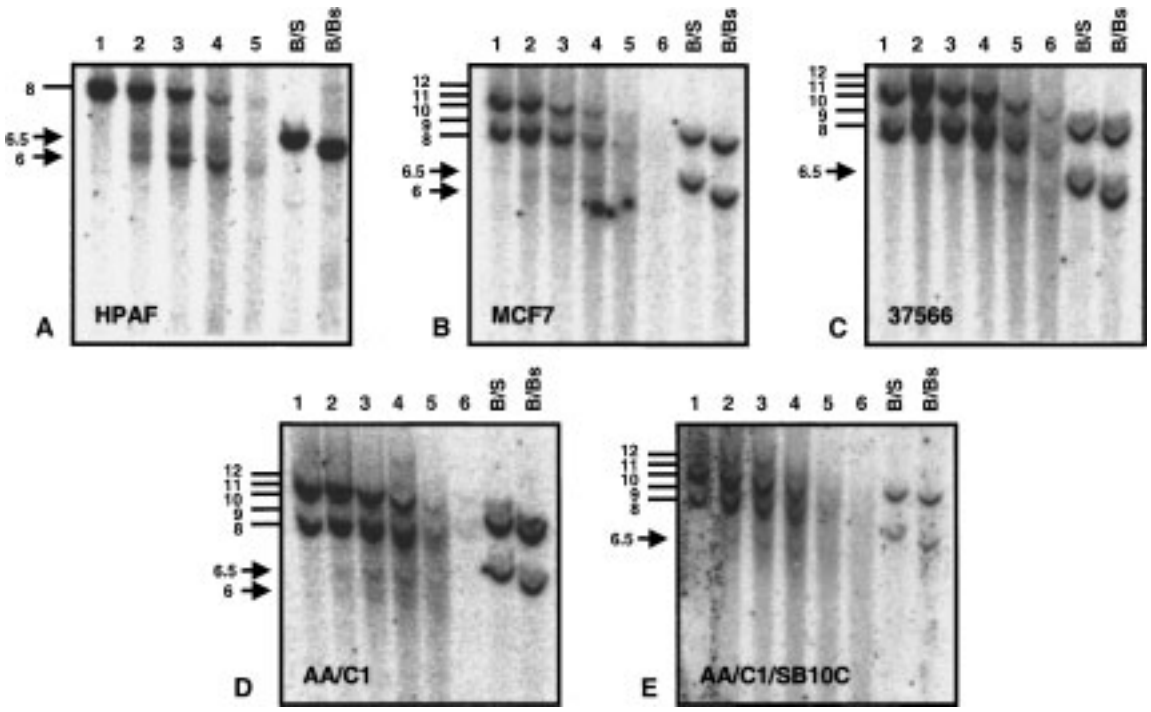

Fig. 3. Detection of the −750 and −250 DHS in chromatin from cell lines. Southern blot of DNase I digested chromatin from **(A)** HPAF, **(B)** MCF7, **(C)** lymphoblastoid (37566), **(D)** AA/C1, **(E)** AA/C1/SB10C cleaved with *Bam*H I and hybridised with 5′MUC1 probe. Lanes 1 (0°C) and 2 (37°C) show DNA prepared from nuclei which had not been treated with DNase I. Lanes 3–6 (Lanes 3–5 in A) show DNA prepared from nuclei treated with increasing amounts of DNase I; (lane 3, 20 units; Lane 4, 40 units; Lane 5, 80 units, Lane 6, 160 units DNase I). Lanes marked B/S and B/Bs denote double digests of the samples in lane 1 of each panel to map the DHS. B = *Bam*H I, Bs = *Bsr*G I, S = *Sac* I. DHS are seen at 6.5 kb in all panels and in A, B and D also at 6 kb.

Identification of DHS in the Human MUC1 Gene in Transgenic Mice

Chromatin was extracted from tissues of the *MUC1* transgenic mice and evaluated for DHS in the human transgene. Figure 5 shows DNase I treated chromatin from transgenic mouse stomach, lung and kidney. A probe specific for human *MUC1* (within intron 1 and part of exon 2 of the gene) hybridised to 3 *Bam*H I fragments in transgenic mice carrying the 10.6 kb *Sac* II fragment of human *MUC1* (Fig. 5A,C,E) instead of the expected single fragment. This suggests there are 2 or more copies of the human gene integrated into these mice. In contrast, a probe specific for the murine *muc1* gene (also within intron 1 and part of exon 2 of the murine gene) hybridised to a single *Bam*H I fragment of about 12 kb, as expected (Fig. 5B,D,F). The human-specific probe used in Fig. 5 is predicted to hybridise to a *Bam*H I fragment of about 9.2 kb based on the restriction fragment map of the human *MUC1* 10.6 kb *Sac* II fragment (11). This is likely to be the smallest of the *Bam*H I fragments, allowing for slight variation between the migration of 1 kb ladder molecular weight markers and DNase I treated, restriction enzyme digested chromatin. Figure 5A,C,E show appearance of the −750 and −250 DHS as 6.5 and 6 kb subfragments of the human *MUC1* genomic fragments. Different DHS (detected as 5.6, 5.0 and 4.6 kb subfragments) are seen with the mouse specific probe, hybridising to the murine *Muc1* gene as shown in panel B. (The mouse-specific DHS are not evident in chromatin from lung and kidney in which the murine *Muc1* gene is expressed, possibly due to insufficient sensitivity to detect chromatin changes in only a small percentage of cells in the tissue sample.) The −250 DHS is more evident than that at −750 bp (Fig. 5A) in chromatin from transgenic mouse stomach. The two DHS are of equal intensity in lung chromatin (Fig. 5C) and in kidney (Fig. 5E). Both DHS were also seen in small intestine and pancreas at low intensity (data not shown).

Expression of the Human MUC1 Gene in Transgenic Mice

Figure 6 shows expression of the *MUC1* transgene and endogenous *muc1* using RT-PCR on RNA from transgenic mice. The primers MUC1HMA and MUC1HMB amplify a fragment of 429 bp from the human gene that is cleaved by *Dra* III into (fragments of 273 and 156 bp) and 424 bp from the murine gene that is cleaved by *Eag* I into 261 and 163 bp fragments. Cleavage of the RT-PCR product with the appropriate enzyme enables discrimination of the human and mouse cDNAs. The assay is not truly quantitative as the PCR reactions are likely to be of unequal efficiency due to a 1 base pair mismatch with the mouse sequence in the

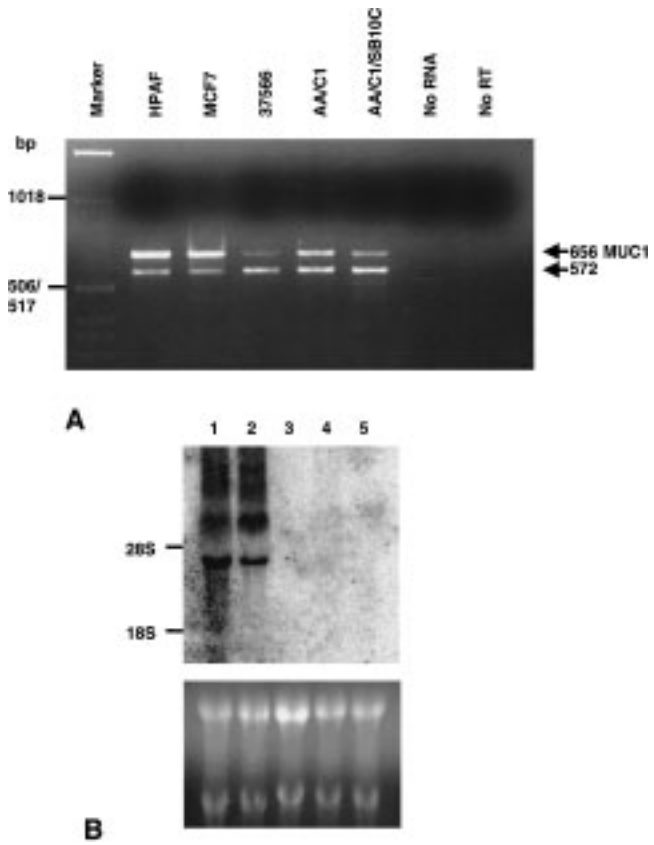

Fig. 4. Expression of *MUC1* in the cell lines analysed in figure 3. (A) RT-PCR using primers for *MUC1* that produce a 656 bp fragment and for β glucocerebrosidase that produce a 572 bp fragment. **(B)** Northern blot of RNA from cell lines, probed with 5′MUC1.

MUC1HMA primer. However, expression of the human *MUC1* gene is seen in lung, stomach, pancreas and kidney (Fig. 6), and also in small intestine, bladder, mammary gland and ovary (data not shown).

Comparison of the Human MUC1 and Murine muc1 Gene Promoters

The 5′ untranslated (X69118: 1851–2941) region of *MUC1* shows approximately 74% identity between human and mouse. Of particular interest are the regions shown in Fig. 7 which correspond to the A) DHS −750 and B) DHS −250. There is high homology between the human and mouse genes immediately 3′ to the −750 DHS which may reflect functional conservation in this region. The −250 DHS region is flanked by regions of high human/mouse sequence conservation both 5′ and 3′ to the site. Since mapping of DHS by Southern blotting is not exact, even with restriction enzyme sites to confirm the locations, these regions of high homology between the human and mouse genes may represent functional importance.

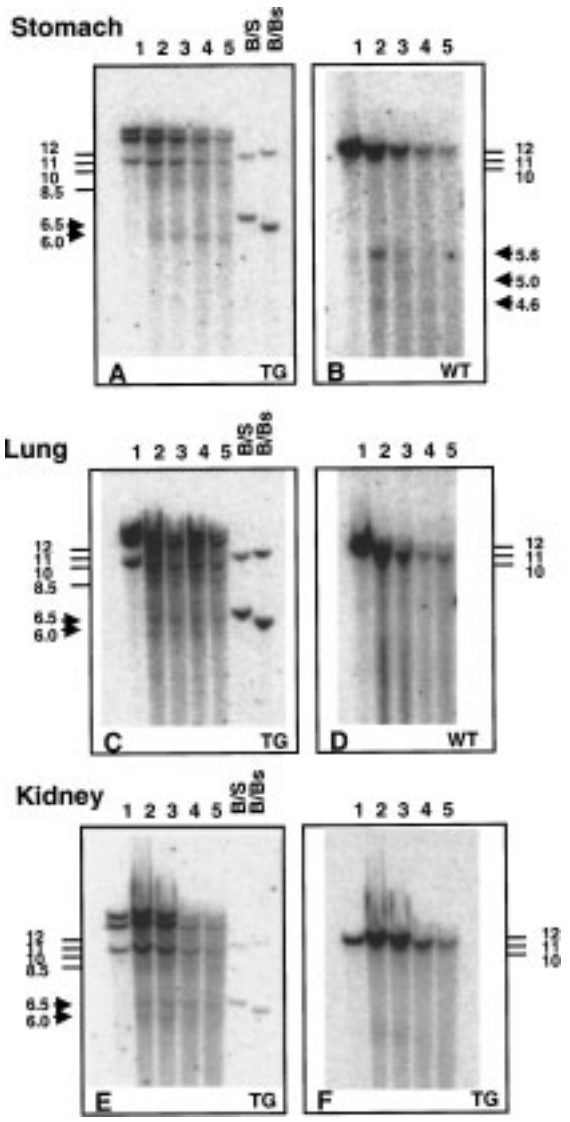

Fig. 5. Detection of the −750 bp and −250 bp DNase I hypersensitive sites in *MUC1* transgenic mice. Southern blots of DNase I digested chromatin from transgenic mouse (TG) or wild type (WT) tissue samples digested with *Bam*H I and hybridised with human specific **(A, C, E)** and mouse specific **(B, D, F)** *MUC1/muc1* probes. For each panel, Lane 1 shows DNA prepared from nuclei which had not been treated with DNase I. Lanes 2–5 show DNA prepared from nuclei treated with a constant amount of DNase I (40U) for increasing lengths of time, 2, 2 min; 3, 5 min; 4, 10 min; 5, 15 minutes. The human-specific probe detects 3 genomic fragments and DHS at 6.5 and 6 kb in chromatin from stomach **(A)** lung **(C)** and kidney **(E)**. The mouse specific-probe detects a genomic fragment of about 12 kb and DHS in stomach only **(B)** at 5.6, 5.0 and 4.6 kb but not in lung **(D)** or kidney **(F)**.

Discussion

DNase I hypersensitive sites in chromatin are often associated with regulatory elements and so can be informative in the identification of control elements that are active *in vivo*. Regulation of expression of the *MUC1* gene has been studied by *in vitro* methods;

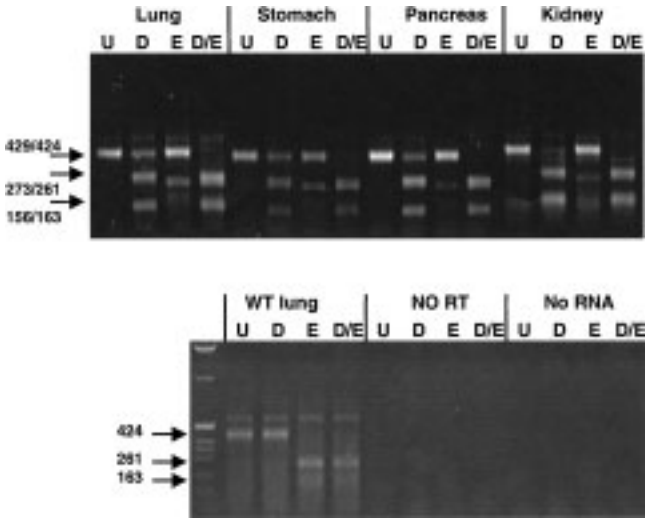

Fig. 6. Detection of the expression of the human *MUC1* gene in transgenic mice. Expression of the human *MUC1* transgene in transgenic mouse lung, stomach, pancreas and kidney. RT-PCR products derived from the human *MUC1* transcript (429 bp) are cleaved with *Dra* III (D) and those from the murine *muc1* transcript (424 bp) are cleaved with *Eag* I (E). U denotes uncut, D/E double digestion with both enzymes; WT wild type C57BL6 mice. No reverse transcriptase (No RT) controls used transgenic mouse stomach RNA as template.

however, the mechanisms that confer tissue specificity on gene expression *in vivo* have not been fully elucidated. We identified two novel DHS in the *MUC1* gene promoter region at −250 and −750 bp with respect to the major transcriptional start site. These sites were first seen in the HPAF pancreatic adenocarcinoma cell line that expresses high level of endogenous *MUC1*.

Further evaluation of these DHS showed that the −750 site was present in many cell types, both epithelial and non-epithelial and its intensity did not correlate with levels of *MUC1* expression. In contrast, the −250 DHS was only seen in cell types that express relatively high levels of *MUC1* and its intensity showed a strong correlation with the abundance of MUC1 mRNA. Of particular interest was the observation that *MUC1* expression levels were reduced and the −250 DHS became undetectable on Southern blots in cells that model the adenoma to carcinoma transition (AA/C1 to AA/C1/SB10C colonic epithelial cell lines).

Detection of DHS in a human gene in transgenic mice can provide strong support for their importance *in vivo*. Mice transgenic for the human *MUC1* gene carried on a 10.6 kb *Sac* II fragment including 5′ and 3′ flanking DNA, show a very similar *MUC1* expression pattern to that seen for the endogenous *muc1* (12). The −750 and −250 DHS were detected in chromatin extracted from certain tissues of these transgenic mice. The relative intensity of the −250 and −750 DHS was not constant, with the −250 DHS being most evident in stomach, though also present in other tissues in which *MUC1* transgene expression was high, such as kidney and lung. These data suggest that the mouse contains the necessary transcription factors to interact with the important sequences generating the DHS. It was of interest to compare the −250 and −750 regions of the mouse and human genes for regions of cross-species homology that might be functionally important. A region of about 34 base pairs of high homology mapped to the predicted core of the −750 DHS. A short region of high homology 5′ and a more extended region immediately 3′ to the predicted location of the −250 DHS were also evident.

These regions of high cross-species conservation were re-evaluated to see if they coincided with important regulatory motifs previously identified in the promoter of the *MUC1* gene. The −750 DHS is close to a predicted half oestrogen response (ER) element and a GC box/Sp1 site (2) though neither of these have been shown to be functionally important in *MUC1* expression.

The −250 DHS corresponds exactly to the location of a region of non-random purine/pyrimidine strand asymmetry (purine/pyrmidine mirror repeat elements-PMR) identified in the *MUC1* promoter (M-PMR2) (26), and is proximal to another PMR element (M-PMR3) located at approximately −130. M-PMR3 is evident in both the human *MUC1* and mouse *Muc1* genes but M-PMR2 is not conserved. These non-random sequences, which contain perfect or nearly perfect mirror repeat elements, may be associated with the formation of H-DNA (intramolecular triple helical) conformations. The M-PMR3 element was shown previously to adopt a relatively uncommon H-DNA conformation (Hy5 isomer) *in vitro* (27). M-PMR2 did not exhibit S1 sensitivity (evidence of H-DNA character) *in vitro* (26) when investigated in a plasmid that contained both M-PMR2 and M-PMR3. However, the M-PMR2 element has not been examined in isolation and is predicted to be capable of forming H-DNA structures under conditions of high superhelical tension. The previous experiments that examined M-PMR2 and M-PMR3 together (26) were conducted under conditions of standard plasmid superhelical density. The energy from superhelical tension that is required for producing H-DNA conformations was probably absorbed by the formation of H-DNA at the M-PMR3 element in those experiments and may not have been sufficient for formation of a second structure at M-PMR2 (which is shorter and may require more energy). Thus, it remains possible that M-PMR2 could form an H-DNA structure *in vivo* under conditions of superhelical density that would be predicted to occur in chromatin upstream of a gene undergoing active transcription. The finding of a DHS at −250 that correlates with transcriptional activity supports the hypothesis that altered DNA conformations in this region are associated with transcriptional activity of the *MUC1* gene. It is also

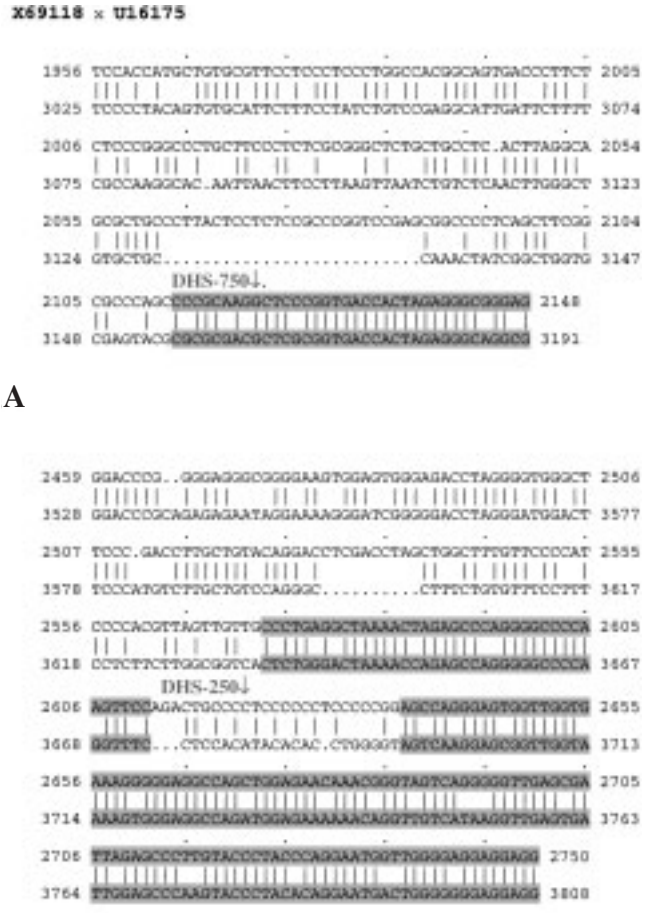

Fig. 7. Comparison of the human *MUC1* and murine *Muc1* gene sequences in the region of the −750 and −250 DHS. (A) The −750 DHS. **(B)** The −250 DHS. Regions of homology between the human (X69118) and murine (U16175) sequences are highlighted.

possible that unknown transcription factors are associated with this element and that these cause the observed DHS. Although the M-PMR3 element was previously shown to not influence transcriptional activity of promoter reporter constructs (28), the M-PMR2 element has not been investigated to date. Hence, the role of M-PMR2 in regulation of *MUC1* gene expression remains intriguing but unclear.

In summary, we have identified two novel DHS in the *MUC1* promoter. One DHS at −750 was detected irrespective of transcriptional activity of the gene. A second DHS at −250 that was associated with transcriptional activity of the gene mapped near previously defined purine/pyrimidine mirror repeat elements that may form intramolecular H-DNA structures. Further elucidation and evaluation of the DNA sequence encompassing the −250 DHS regions is warranted to elucidate the mechanisms of action of potential regulatory elements located within this region of the *MUC1* promoter.

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